

THERMAL ALTERATION OF ORGANIC MATTER IN RECENT MARINE SEDIMENTS

II. ISOPRENOIDS¹

R. Ikan^a, M.J. Baedeker and I.R. Kaplan

Department of Geophysics and Planetary Physics

University of California

Los Angeles, California 90024

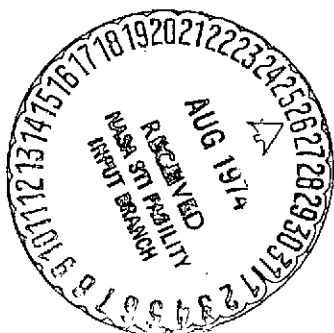
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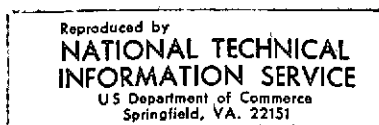
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¹ Publication No. 1244: Institute of Geophysics & Planetary Physics
University of California, Los Angeles,
California 90024

^a Permanent address: Department of Organic Chemistry,
Natural Products Laboratory, Hebrew University
Jerusalem, Israel

ABSTRACT

A series of isoprenoid compounds were isolated from a heat-treated marine sediment (from Tanner Basin) which were not present in the original sediment. Among the compounds identified were: phytol, dihydrophytol, C_{18} -isoprenoid ketone, phytanic and pristanic acids, C_{19} - and C_{20} -monoolefines, and the alkanes pristane and phytane. The significance and possible routes leading to these compounds is discussed.

INTRODUCTION

Ever since Bendoraitis et al. (1962) first isolated pristane (2,6,10,14-tetramethylpentadecane) from crude oil in concentrations equal to 0.5% of the total, there has been considerable discussion on the origin of the isoprenoid hydrocarbons in sediments and in petroleum. The two major isoprenoid hydrocarbons normally detected are pristane and phytane (2,6,10,14-tetramethylhexadecane), although C_{18} , C_{16} and C_{15} alkanes are also common in oil shales and crude oil, and in some environments, these become the dominant branched hydrocarbons (Arpino et al. 1972). Bendoraitis et al. (1962) believed that the starting compound was chlorophyll from which the phytol side chain is cleaved and subsequently oxidized to an acid and finally decarboxylated to the C_{19} alkane. Support for a phytol origin had come from studies of Bayliss (1968), who heated algae and grass and showed that both pristane and phytane formed as products. Subsequent experiments have been performed by heating phytol in the presence of montmorillonite (Arpino 1972) and in the presence of bentonite (Eglinton 1972) producing a series of C_{14} to C_{20} isoprenoid alkanes.

Blumer and co-workers have isolated pristane, phytadienes and a variety of C_{19} monoolefins from marine organisms (Blumer and Thomas 1965a; 1965b; Blumer et al. 1971), particularly zooplanktonic copepods. By feeding algae on which phytol- $U-^{14}C$ was adsorbed to calanid copepods. Avigan and Blumer (1968) were able to isolate ^{14}C -tagged phytanic acid and pristane as major products. Phytane was not detected in marine animals by the above authors.

Extraction of recent marine sediment have yielded small quantities (< 50ppb) of pristane and either no phytane (Blumer

and Snyder 1965) or only trace quantities, generally less than the pristane (Brown et al. 1972). Avigan and Blumer (1968) believed that zooplanktonic copepods may be an important source of pristane both in the marine food chain and in sediments. Hodgson et al. (1967) have reported finding pristane and also phytane in land plants, which led Hodgson et al. (1968) to comment that they are probably not formed in the sediment by diagenetic processes through the alteration of the phytol ester of chlorins.

Several recent studies, however, suggest that certain isoprenoid compounds so far not detected in marine organisms, may be forming in the sediment column from phytol, either during early diagenesis or during maturation of sediment as a result of lithification and thermal alteration. These include dihydrophytol (Sever and Parker 1969), C_{18} isoprenoid ketone (6,10,14-trimethylpentadecan-2-one; Ikan et al. 1973; Simoneit 1973), and the C_{20} alkanes referred to above. It had been first postulated by Curphey (1952), that phytol could be converted by oxidation to a ketone of 2 less C atoms and subsequently reduced to the alkane.

Maxwell, et al. (1972, 1973) and Cox et al. (1972a) have given evidence based on stereochemical information that phytane and its intermediates have formed from the phytol ester of chlorophyll at relatively low temperatures, probably during early diagenesis. Based on this information and other data from their laboratory and published results, Cox et al. (1972b) proposed a scheme for the transformation of phytol into the C_{18} and C_{20} isoprenoid alkanes. The pathway they outlined differs from that previously suggested by McCarthy and Calvin (1967) and relies on intermediates

which at that time had not been isolated and identified.

The present investigation undertook a study on the transformation of chlorins in an organic-rich (~ 12% org. C) marine sediment from Tanner Basin, on the outer shelf of the California Borderland (see Emery 1960. The mildly-reducing sediment from a 2 m core was homogenized and heated at different temperatures over a time period from 7 to 64 days. In this report, we describe the isoprenoid compounds isolated, and suggest a pathway which may be followed during alteration of the phytyl side chain of deposited chlorophyll to the end products, pristane and phytane.

METHODS

Fifty-gram samples of sediment from Tanner Basin were sealed, under nitrogen, in thick-walled glass tubes and subjected to heat treatment at temperatures of 65°, 100° and 150° for varying periods of time (7, 30 and 64 days).

Extractable organic material was removed from the sediment with benzene-methanol according to the method of Brown et al. (1972). A crude separation of the extract was achieved by column chromatography on silicic acid using the following eluents: hexane, benzene and methanol. Branched hydrocarbons, in the hexane fraction, were separated from the normals by 5Å molecular sieves. The isoprenoid olefins and alkanes were identified (Fig. 1), and their concentrations are listed in Table 1. Portions of the benzene and methanol fractions from silicic acid chromatography were combined and refluxed in methanolic KOH and the

alcohols and fatty acids separated (Aizenshtat et al. 1973). The isoprenoid alcohols, dihydrophytol, phytol and pristanic and phytanic acids were identified. A C_{18} -isoprenoidal ketone (6,10,14-trimethylpentadecan-2-one) was also isolated during these experiments (Ikan et al. 1973). Identification of these compounds was based on GC retention time data and on GLC-MS measurements and, in all cases, comparisons were made with authentic compounds.

RESULTS

As stated above, a series of isoprenoid compounds were isolated from the heated sediment which were not identified in the original sediment. These include the alcohols phytol and dihydrophytol, the C_{18} isoprenoid ketone, phytanic and pristanic acids, the C_{19} monoolefine pristene-1 (norphytene-1), and pristene-2 (norphytene-2) and the C_{20} monoolefines phytene-1 and phytene-2, and the alkanes pristane and phytane. We did not detect phytdienes, which are a common constituent of zooplankton (Blumer and Thomas 1965a). Data for 6,10,14-trimethylpentadecan-2-one are given elsewhere (Ikan et al. 1973).

From Table 1, it can be seen that there is a direct correlation between time of heating and amount of the isoprenoid hydrocarbons formed. Only small amounts of C_{19} hydrocarbons form at 100°C within 64 days of heating, and then only one isomer of the olefin (pristene-1) was observed. Two isomers of the unsaturated hydrocarbon phytene-1 and phytene-2, as well as the alkane phytane, were measured in sediment heated to 100°C, the amounts increasing approximately linearly with time of heating. At 150°C, the results

appear to be more complex. Here, C_{19} , as well as C_{20} alkanes, appear to increase with increasing time. Pristane shows the greatest relative increase with time. The olefins indicate an increase in concentration when heating has occurred to 30 days, but with both pristene and phytene, one isomer decreases in content and the other increases when heating has continued for 60 days. The overall trend, however, is for increase in total hydrocarbon content with time of heating. Although they are in lower concentration, the alkanes are forming more rapidly than the alkenes (Fig. 2).

Because of lower concentration and less sensitivity in detection levels, the isoprenoid alcohols and acids could not be measured quantitatively in the sequence of reactions which were run at the various temperatures and times. Hence, several reaction vessels with Tanner Basin sediment were sealed and left for a period of 30 days at 100°C and 150°C , respectively. Table 2 indicates that the content of both the alcohol and acid is greater at the lower temperature. It is therefore obvious that these moieties are less stable than the hydrocarbons.

DISCUSSION

A scheme for the alteration of phytol, which is presumably produced from hydrolysis of chlorins, is shown in Fig. 3. All the intermediate compounds leading to formation of the alkanes, with the exception of the unsaturated phytenic acid, have been isolated in this study. This acid has been reported as a constituent of triglycerides in animal lipids (Baxter et al. 1967), but has not been reported in recent or ancient sediments. We believe, therefore, that it is an unstable intermediate and rapidly decomposes to pristene by decarboxylation.

Under mild oxidizing conditions, phytol should be transformed either to the C_{18} ketone or to phytanic acid. Under reducing conditions, phytol will first be hydrogenated to dihydrophytol. Phytol is an uncommon constituent of recent marine sediments (Sever and Parker 1969; Aizenshtat et al. 1973) and therefore the phytol group of chlorins is either rapidly oxidized, or reduced during early diagenesis.

During the present experiments, the phytol content at 100°C was less than half the dihydrophytol content and $1/4$ of the ketone content. At 150°C , the ratio even drops further to $1/4$ and $1/12$, respectively.

Whereas the hydrocarbon content increased with time and temperature of heating, the total alcohol and acid contents decreased (Table 2). This may be direct evidence for the conversion of these polar compounds to the non-polar and more stable hydrocarbons, by dehydration and decarboxylation reactions.

It is apparent that both oxidizing and reducing reactions occur during the heating of the sediment. In the reducing reactions, phytol will be converted to phytane via dihydrophytol and phytene. Under oxidizing conditions, phytol can be degraded either to the C_{18} ketone or to phytanic acid. It is apparent that in the oxidative pathway there is competition between the C_{18} and the C_{19} series of alteration. The reducing pathway would preferentially lead to formation of phytane. Because the sediment under consideration is reducing, phytane should be the dominant alkane formed, and this was observed (Table 1).

The scheme outlined in Figure 3 was arrived at from inter-

pretation of the most feasible reactions which could occur, based on a knowledge of the compounds formed and changes which occur during the heating experiments performed here. No confirmatory trials were undertaken using C^{14} -tagged compounds. It is probable that under somewhat different conditions, catalytic reactions may lead to formation of other intermediates not recognized in this study. One such compound is probably phytadiene (Simoneit and Burlingame 1973). Furthermore, depending on the clay-organic interactions, a variety of isomers may be formed in different ratios to those measured in the monoolefins isolated in the present study. The reaction scheme shown in Fig. 3, may therefore not necessarily be unique in all its detail.

The distribution patterns of the isoprenoid alkanes in sediments, oil shales and crude oils present an interesting dilemma to organic geochemists. It is generally believed that they originate from the degradation of the phytol ester of chlorins, during early diagenesis. However, isoprenoid moieties have only been detected in trace amounts in recent unlithified marine sediments, and until recently dihydrophytol, pristane and phytane were the only compounds recognized. Other intermediates in the break-down pattern of phytol to isoprenoid alkanes had not been detected in recent sediments, and there was some question as to the origin of these alkanes.

The present studies confirm the previous postulates, that phytol in sediments, generated during alteration of chlorophyll, is probably a major source of isoprenoids. In this investigation, several of the intermediates necessary to complete the pathway were isolated, with the exception of phytenic acid. Our results also probably confirm the explanations of Brooks et al. (1969)

and of Powell and McKirdy (1973), on the significance of pristane to phytane ratios in crude oil. The predominance of the former in continental petroleum, may be due to exposure of the organic matter to an oxidizing environment during deposition and early diagenesis.

Several major problems still exist in explaining the distribution of the isoprenoid hydrocarbons. Although C_{19} and C_{20} alkanes are the most common, the range extends from C_{14} to C_{25} alkanes. There is presently no explanation to the complex distribution pattern observed in certain shales and crude oils (Ikan and Bortinger 1971; Han and Calvin 1969). It should be mentioned in passing that although chlorophyll may be the major source for isoprenoid hydrocarbons in the vast majority of marine and continental sediments, in highly saline lakes, the halophilic microorganisms contain a phosphatidyl glycerophosphate lipid (Kates et al. 1965), which on decomposition degrades to phytane and other C_{20} isoprenoid compounds (Kaplan and Baedeker 1970).

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FIGURE CAPTIONS

Figure 1: Branched hydrocarbons chromatographed on a 5'X 1/8" column packed with 3% Apiezon L on 100/120 mesh Gas Chrom Q, programmed from 100° to 300° at 4°/min. Compounds labeled are: (a) α -ionene (b) pristane (c') pristene-1 (c) pristene-2 (d) phytane (e) phytene-1 (f) phytene-2.

Figure 2: Concentration of isoprenoid hydrocarbons vs. time of heating.

Figure 3: Pathways for diagenesis of chlorophyll.

Table 1

Concentration in ppm of isoprenoid hydrocarbons
in heat-treated Tanner Basin sediments

	Pristane	Pristene-1 ^a	Pristene-2 ^{b±}	Phytane	Phytene-1 ^c	Phytene-2 ^c
100°C/7 da.		0.07	tr.	0.06	0.25	0.37
100°C/30 da.	tr.	0.13	tr.	0.07	0.44	0.78
100°C/64 da.	tr.	0.40	tr.	0.17	0.92	1.45
150°C/7 da.	0.07	1.04	0.34	0.40	1.84	2.55
150°C/30 da.	0.13	1.39	0.82	0.64	2.57	3.42
150°C/64 da.	0.65	0.68	3.30	1.98	3.98	3.38

a. 2,6,10,14 tetramethylpentadec-1-ene

b. 2,6,10,14 tetramethylpentadec-2-ene

c. 3,7,11,15 tetramethylhexadec-1-ene

d. 3,7,11,15 tetramethylhexadec-2-ene

± = tentatively identified on basis of GLC-MS data. The monolefins, pristene-1 and pristene-2, are usually referred to as norphytenes.

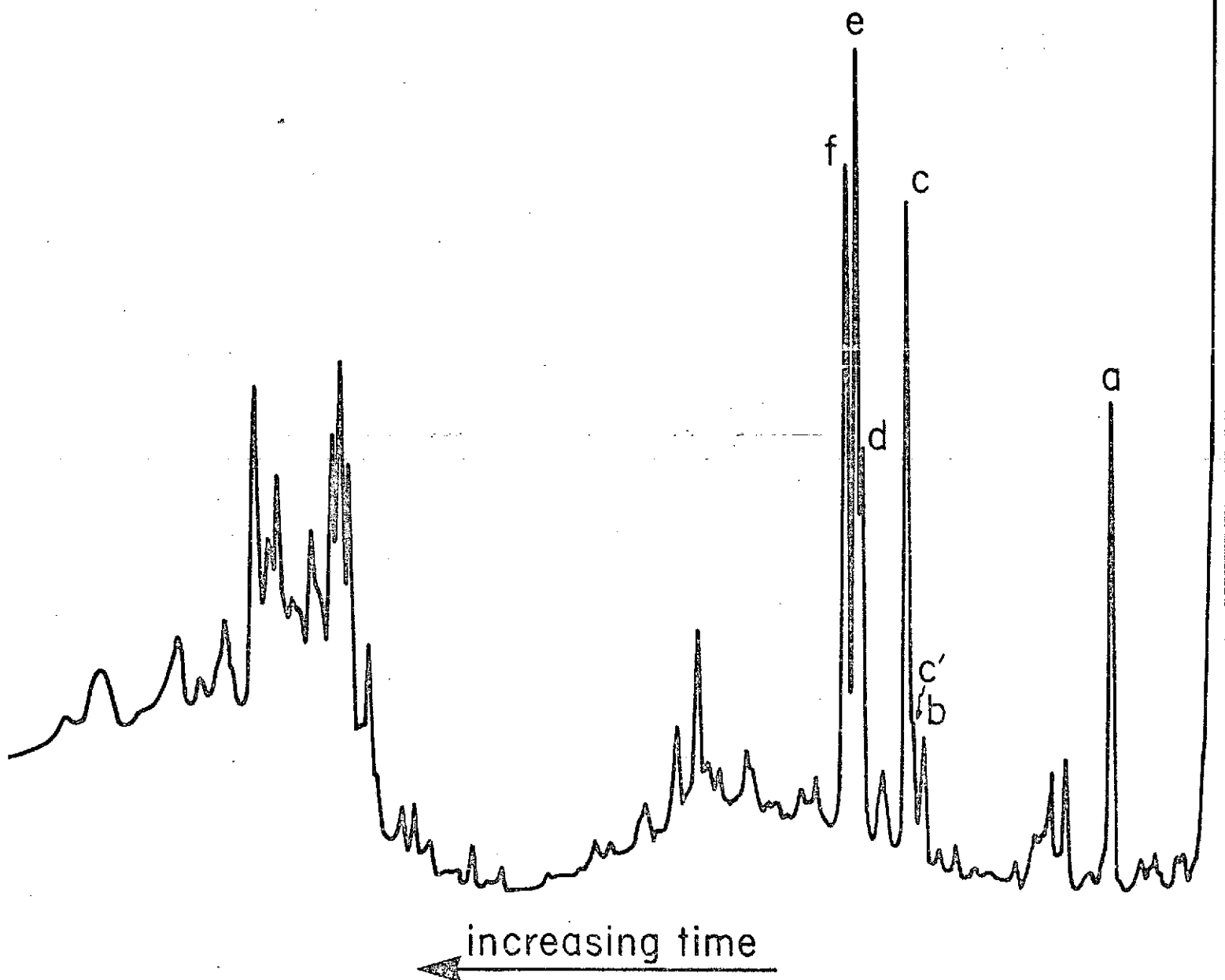
The alkene isomers had the same mass spectra, therefore assignment of the double bond was made by comparison with the results of Blumer and Thomas (1965) who showed the -1-ene has a lower retention time than the -2-ene.

Table 2. Formation of isoprenoid alcohols and acids during heating sediment for 30 days at 100° and 150°C

Compound (ppb)	Temperature	
	100°C	150°C
Phytol	374	142
Dihydrophytol	803	573
DiHPL/PL	2.2	4.1
Phytanic acid	110	38
Pristanic acid	58	18
PHA/PRA	1.9	2.1

Tanner Sediment
heated 150°-60 days

Hydrocarbons



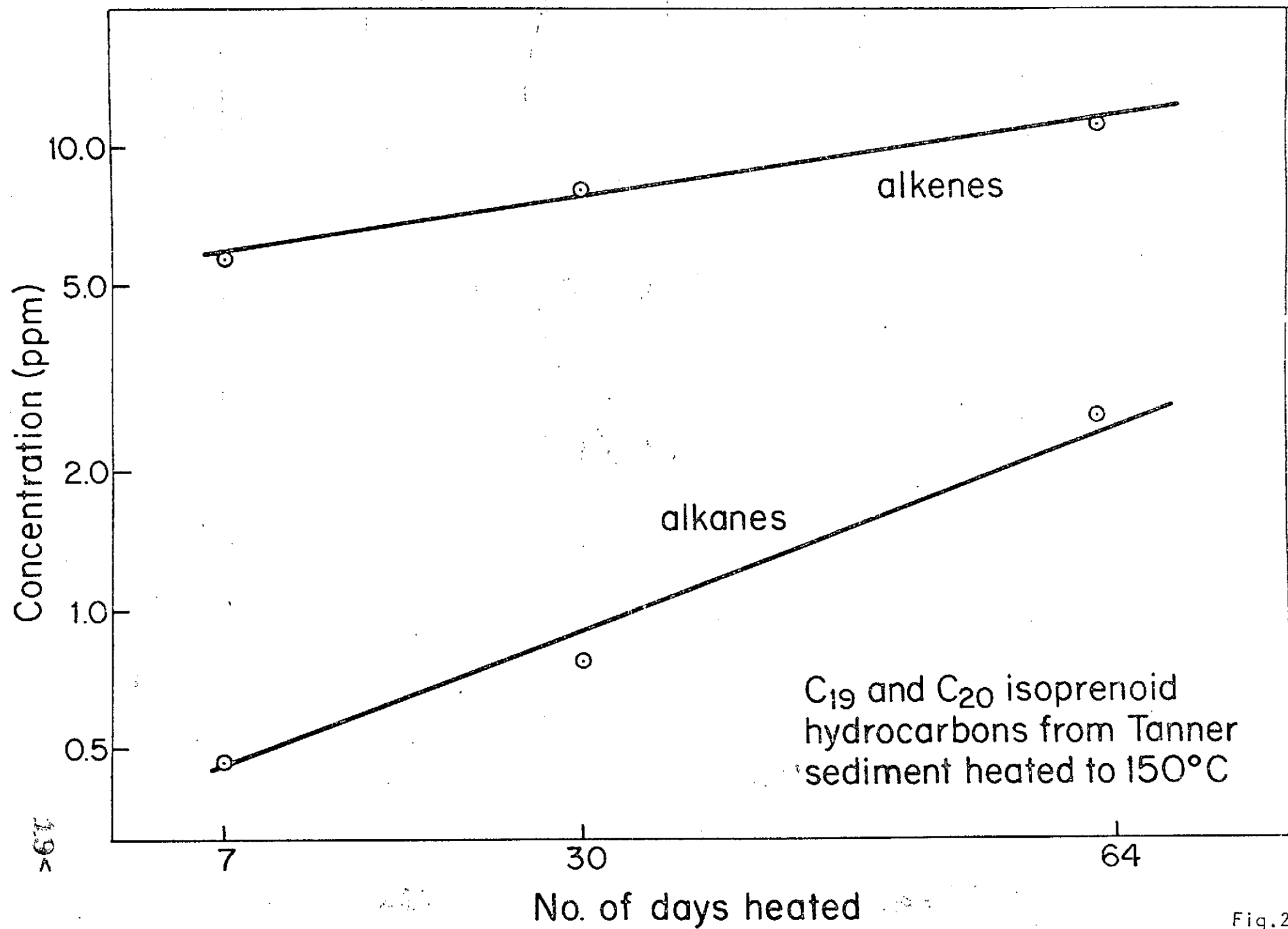


Fig.2

THERMAL ALTERATION OF ORGANIC MATTER IN RECENT
MARINE SEDIMENTS

III. ALIPHATIC AND STEROIDAL ALCOHOLS¹

R. Ikan^a, M.J. Baedeker and I.R. Kaplan

Institute of Geophysics and Planetary Physics
University of California
Los Angeles, California 90024

¹Publication No. 1245: Institute of Geophysics and Planetary Physics
U.C.L.A., Los Angeles, California 90024

^a Permanent address: Department of Organic Chemistry,
Natural Products Laboratory, Hebrew University
Jerusalem, Israel

ABSTRACT

Recent sediments from Tanner Basin and Bandaras Bay have been analyzed for normal, isoprenoid and steroidal alcohols using chromatographic (column, GLC, TLC), and spectroscopic (UV, IR, MS) methods prior to and after heat-treatment (from 65° to 150°C). Normal saturated alcohols (C_{14} - C_{24}) and some monounsaturated alcohols were identified, as well as the isoprenoid alcohols, phytol and dihydrophytol.

Two series of sterols (Δ^5 and Δ^7) were found in Tanner Basin, and Δ^5 -sterols and triterpenes, in Bandaras Bay sediment. Sterols from both sediments also contained the corresponding stanols. GLC and MS study of branched-cyclic hydrocarbons revealed the presence of steranes and sterenes; the latter being intermediates in sterane formation.

INTRODUCTION

Normal, branched-chain and isoprenoid alcohols are abundant in nature, however, their distribution in recent and ancient sediments has been infrequently reported (Sever and Parker, 1969; Hoering 1967; Aizenshtat et al. 1973; Cox et al. 1972). Most of the alcohols identified in recent sediments are straight-chained and saturated, ranging from C_{12} to C_{28} . Some unsaturated alcohols have also been detected in recent muds, however, they are absent in ancient sediments.

Sterols are minor constituents of plant and animal kingdoms. They are distributed as free compounds or esters of higher aliphatic acids. They have been isolated from both recent and ancient sediments. Thus, Attaway and Parker (1970) have identified a variety of sterols in a 2,000-year-old sediment. Henderson et al. (1972) have identified sterols in a Pleistocene brackish lake sediment, and Simoneit and Burlingame (1972) found steroidal compounds in Pacific Ocean sediments from the JOIDES Deep Sea Drilling Project. Mattern et al. (1970) have isolated sterols, including 4-methyl-sterols from the Eocene Messel oil shale of Germany.

The presence of a series of steranes, corresponding to sterols, and also triterpenes have been reported in marine and terrestrial sediments (Henderson et al. 1971 ; Ikan and Bortinger 1971). Production of steroidal hydrocarbons under maturation-simulating conditions were reported by Steel et al. (1972) and Rhead et al. (1971).

The purpose of this study was to compare the distribution of normal and isoprenoidal alcohols, sterols and steranes in natural and heat-treated marine sediment in order to understand the diagenetic processes these compounds undergo.

EXPERIMENTAL

Separation Procedures

A description of the environments is given in Ikan et al. (1974). Approximately 50 g samples of Tanner Basin sediment were sealed in glass bombs and heated at 65°, 100° and 150° C for periods of 7, 30 and 64 days. One sample was freeze-dried, then subjected to heat treatment at 150°C for 64 days. A sample of Bandaras Bay mud was heated to 150°C for 7 days. Standard samples (untreated mud) were also analyzed. Extraction and separation procedures employed have been given elsewhere (Aizenshtat et al. 1973). Crude separation was achieved by applying the concentrated extract to a silicic acid column and eluting with (1) hexane, (2) benzene and (3) methanol.

Most alcohols and sterols were detected in the methanol fraction and only small amounts were found in the benzene fraction. The two fractions were concentrated in vacuo, methanolic KOH was added, and the solution refluxed for two hours. Methanol was then evaporated in vacuo and replaced with water. The non-saponifiable fraction, containing the alcohols and sterols, was extracted with hexane:benzene (9:1). Part of this fraction was converted into acetyl derivatives by refluxing with acetic anhydride for two hours.

Steroidal hydrocarbons were identified in the hexane fraction of the silicic acid column. Branched and cyclic hydrocarbons were separated from the normals by refluxing with 5 Å molecular sieves.

Due to the lower abundance of normal and especially isoprenoidal alcohols and the lower level of sensitivity on analysis by gas chromatography, two larger samples (100 g) of Tanner sediment were processed, one at 100°C for 30 days and the other at 150°C for 30 days. The alcohols were separated according to the procedure above, except an additional step of urea adduction (Douglas et al. 1971) was included to separate normal and branched components.

Sterols, aliphatic alcohols and steroidal hydrocarbons were identified using the techniques of gas chromatography, mass spectroscopy, and thin-layer chromatography.

Thin-Layer Chromatography

The free sterols were spotted on (0.25 mm) silica gel G plates and developed with petroleum ether-ethyl ether-acetic acid (20:4:1). Examination under UV light (254 and 360 nm) before and after spraying with 50% sulfuric acid, and charring, revealed sterols as brown spots (Rf 0.64), and triterpenes as a pink spot (Rf 0.72). The latter was observed in the Bandaras Bay sample only.

Silica gel plates impregnated with silver nitrate were prepared as described by Ikan (1965). Samples of sterol acetates (in hexane) were spotted on the starting line of the plate and developed with light petroleum-ethyl-acetate (98:2). Spraying

the plates with 50% sulfuric acid and charring, revealed (in 360 nm light) steryl acetates as brown spots, $R_f = 0.37$, and stanyl acetates as sky-blue fluorescing spots, $R_f = 0.43$.

Aliquots of branched hydrocarbon fractions were applied on silver nitrate-impregnated plates and developed with hexane-toluene (18:1). Spraying with 50% sulfuric acid followed by charring, revealed 5 α -cholestane and cholestene as brown spots, R_f values 0.94 and 0.50, respectively.

Catalytic Reduction

An aliquot of alcohol acetates was added to a pre-reduced Adam's catalyst (PtO_2) in methanol, and hydrogen was bubbled through the solution for two hours. The catalyst was filtered off and the solution concentrated and re-examined by gas chromatography.

Instrumentation

Aliphatic alcohols and sterols were analyzed as acetates by gas chromatography and the retention times compared with known compounds. The samples were chromatographed on a 5 ft. X 1/8 inch stainless steel column packed with 3% OV-101 on 100-120 mesh Chromosorb Q, programmed from 100° to 280°C. The same column was coupled with a CEC 21-491 mass spectrometer to obtain mass spectra of individual compounds.

Steroidal hydrocarbons were analyzed on a 100 ft. (0.01 I.D.) stainless steel capillary column coated with Ap.L.

Ultraviolet spectra were obtained on a Cary 15 spectrophotometer equipped with a 0.1 cc microcell. Infrared spectra

were obtained on Perkin-Elmer 137 spectrometer.

RESULTS

Aliphatic alcohols

The concentration of normal alcohols in both the Bandaras Bay and Tanner Basin sediments was increased on heating. The alcohols were initially present in Bandaras Bay sediment in amounts of 250 ppb and after heating at 150°C for 7 days, their concentration increased to 1000 ppb. Approximately 16% of the total in the heat-treated sediment was due to unsaturated compounds (mainly C_{18} , C_{20} , C_{22} , and C_{24}). In the Tanner sediment, the alcohol content increased from 1900 ppb in the untreated mud to 4300 ppb in a sample heated to 100°C for 7 days. The major alcohols in the heat-treated sample were the mono-unsaturated C_{22} and C_{24} compounds with smaller amounts of C_{20} , C_{18} , C_{26} and C_{16} analogues (in order of decreasing abundance), which amounted to 55% of the total straight-chained alcohols.

Table 1 shows changes in the major compounds, Δ - C_{22} and Δ - C_{24} , with increasing time and temperature. The maximum yield of unsaturated alcohols was obtained by heating the samples at 150°C for two months.

Although normal alcohol content of Bandaras Bay sediment appears to be lower than in Tanner Basin sediment, the ratio of alcohol to organic carbon is greater in heat-treated samples from Bandaras Bay (5×10^{-7}) than that from Tanner Basin (3×10^{-7}). As these n-alcohols are present in the untreated sediment, heating probably causes the release of these compounds, from a bound

matrix. There is no evidence to indicate that the normal alcohols have been formed during heat treatment.

Figure 1A shows a GLC of the total alcohol fraction (chromatographed as acetates) isolated from Tanner sediment heated to 100°C for 7 days. Figure 1B shows the same sample after hydrogenation. The unsaturated compounds Δ -C₂₀, Δ -C₂₂ and Δ -C₂₄ (e,h,j in Fig. 1A) were reduced as shown in Fig. 1B. GLC-MS measurements of alcohol acetates revealed the presence of normal alcohols (both saturated and unsaturated). Because the alcohols were chromatographed as acetates, the ion of highest m/e in each mass spectrum was M-60. In general, the mass spectra of long-chained alcohols resembled the corresponding hydrocarbons with peak groups 14 amu apart. Thus, the peaks h and j (Fig. 1A) of the chromatograph correspond to mono-unsaturated C₂₂ and C₂₄ alcohols with highest m/e's of 306 and 334, respectively.

Because the isoprenoids phytol and dihydrophytol could not be differentiated under these conditions from n-C₁₈ and Δ -n-C₁₈ alcohols, respectively, a larger sample was separated into normal and branched components by urea adductation. A GLC-MS of the branched fraction verified the presence of phytol and dihydrophytol (Table 2). Neither of these isoprenoids could be identified in the untreated mud, but were present in sediment treated at 100°C and 150°C for 30 days. Their amounts decreased in the 150°C sample (Table 2).

Sterols

Analysis of sterol fractions from natural and heat-treated Tanner Basin sediment by thin-layer chromatography, revealed the

presence of sterols and stanols (ratio of 1:1) and the absence of triterpenes. However, Bandaras Bay sediment does contain some triterpenes, in addition to sterols and stanols. Data for identified sterols are summarized in Table 3, whereas the distribution of cholesterol, the major sterol, after thermal alteration, is presented in Table 4.

It should be pointed out that the "critical pairs" of sterols and stanols could not be resolved under the GLC conditions employed (Henderson et al. 1971 ; Goad et al. 1972), they could, however, be separated by the thin-layer argenation technique, where the saturated sterols have higher R_f values than the corresponding unsaturated ones.

The ultraviolet spectrum of the steroidal mixture of Tanner Basin showed the typical Δ^5 -sterol absorbance at 225-230 nm and had an unusually high end absorption with a maximum at 215 nm, which is characteristic of Δ^7 -sterols (Blandon et al. 1952; de Souza and Nes 1968). This was corroborated by the infrared spectrum which showed a frequency of 1030 cm^{-1} for Δ^7 -sterols. The infrared spectrum also, had a weak band at 968 cm^{-1} corresponding to some trans- Δ^{22} -sterols (Dobriner et al. 1953). The ultraviolet spectrum of the steroidal Bandaras Bay fraction displayed only one peak at 225-230 nm, characteristic of Δ^5 -sterols.

The sterols were chromatographed along with the aliphatic alcohols as acetates on an OV-101 column.. Figure 1B corresponds to the same sample after catalytic reduction, and shows the increase of saturated steroidal alcohols (n,p,r).

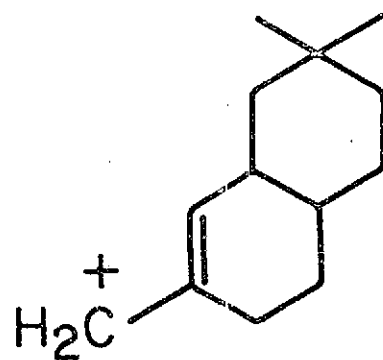
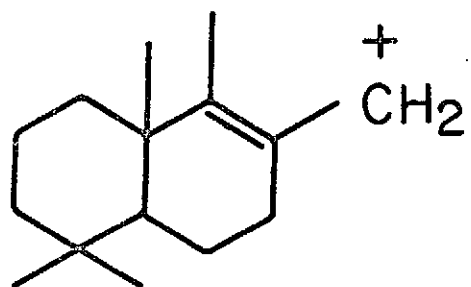
Mass spectral data supports the tentative identifications of sterols which were based on chromatographic (GLC, TLC) and

spectroscopic (IR,UV) results. The GLC of Tanner Basin steryl acetates showed six components (Figure 1A). The mass spectra of these compounds had highest m/e peaks (M-60) at 366, 368, 380, 382 (2 compounds) and 396, respectively (Table 3). The identity of Δ^7 -ergosterol was confirmed by the presence of a weak peak at m/e 382 (M-60; loss of acetate) and 367 (M-75; loss of acetate, plus methyl). (Gupta and Scheuer 1968). GLC of Bandaras Bay steryl acetates showed five peaks corresponding to cholesterol, brassicasterol, campesterol, stigmasterol, β -sitosterol and a triterpene, the latter two appearing as one peak. The corresponding (M-60) peaks in the mass spectrum were: 368, 380, 382, 394, 396 and 426 molecular ion peak corresponding to a triterpene. Peaks which appeared at m/e 191, and 149 are characteristic of pentacyclic triterpenoids. The peak at m/e 191 may arise from one of the following fragments of a pentacyclic triterpene, such as lupeol.

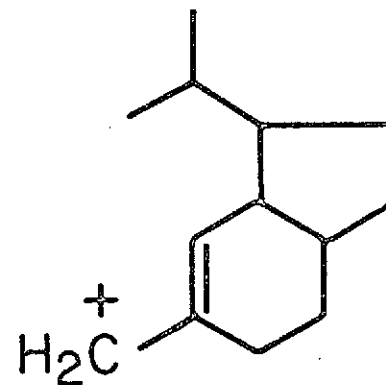
(Scheme 1.)

Thin-layer chromatography of the branched hydrocarbons of Tanner Basin revealed the presence of 5 α -cholestane and cholestene. Gas chromatographic results also allowed the tentative identification of 5 α -, 5 β -cholestane and cholestene.

The mass spectrum showed the characteristic strong peak of steranes at m/e 217, which is probably due to the following tricyclic fragment:



m/e 191



(Scheme 2)

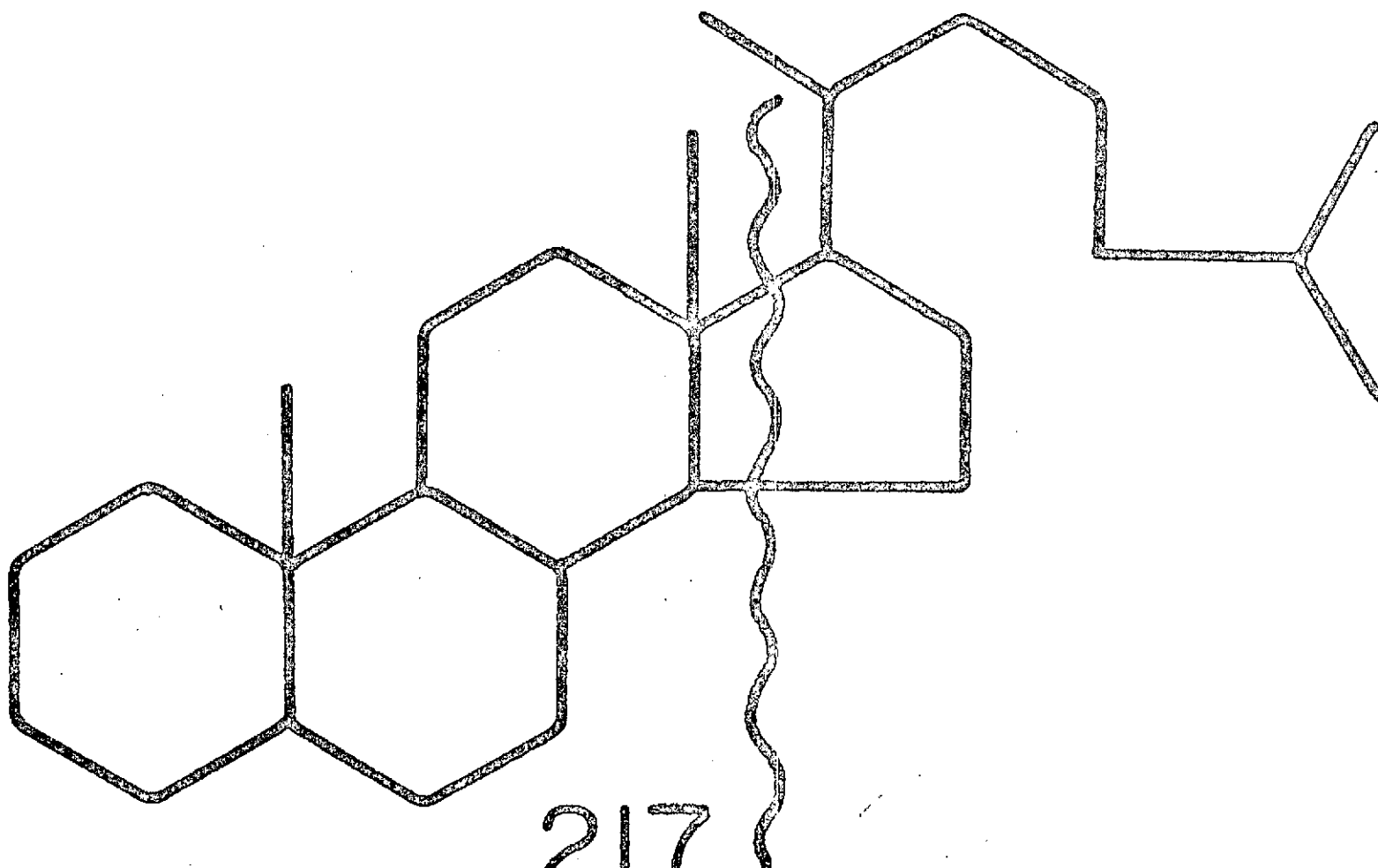
The molecular ion peaks that appeared at m/e 372, 386 and 400 correspond to cholestane, ergostane and stigmastane and those at 370, 384 and 398 to the corresponding sterenes.

DISCUSSION

Alcohols

Alcohols detected in Tanner Basin probably have originated from wax esters. It has been reported that the alcoholic components of plant wax esters are straight-chained and saturated (Kollattukudy 1970); however, in a large variety of marine organisms, the alcohols (from wax esters) are either saturated or mono-unsaturated (Nevenzel 1970). Thus, the biota might explain the high concentration of mono-unsaturated $n-C_{22}$ and $n-C_{24}$ alcohols found in the Tanner sediment.

The C_{20} -isoprenoid alcohols, phytol and dihydrophytol which were identified in this study, probably formed from hydrolysis of pheophytin and dihydropheophytin. Dihydrophytol was more abundant than phytol in the samples analyzed and was also more stable. The ratio of dihydrophytol/phytol was 2.2 and 4.1 in samples heated to 100° and 150°C, respectively. Phytol has been infrequently identified in recent sediments (Nissenbaum et al. 1972), however, dihydrophytol has been reported in samples from several environments (Sever and Parker 1969). It has been shown that phytol is probably oxidized to C_{18} isoprenoid ketone in the



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sediment (Ikan et al. 1973). This probably occurs during early diagenesis. Under anaerobic conditions, phytol is probably converted to dihydrophytol by hydrogenation of the double bond. Hence, there appears to be relatively rapid degradation of the phytyl ester by both oxidative and reductive processes.

Sterols

Two series of sterols (Δ^5 and Δ^7) found in Tanner Basin strongly suggest that they originated in the marine environment, and underwent partial reduction during sedimentation. Indeed, such sterols as cholesterol, 22-dehydrocholesterol, brassicasterol, campesterol, β -sitosterol and Δ^7 -ergosterol have been found in marine organisms (Ikekawa et al. 1968; Gupta and Scheuer 1968; Patterson 1971). Presence of Δ^5 -sterols and a trimethyl steroid (triterpene) in the Bandaras Bay sediment, indicates a contribution from a terrestrial source.

It was not possible to ascertain the absolute stereochemistry of the side chain of sterols because of the small content available. Therefore, the presence of both, the 24S- and 24R-epimers could not be excluded (Patterson 1971).

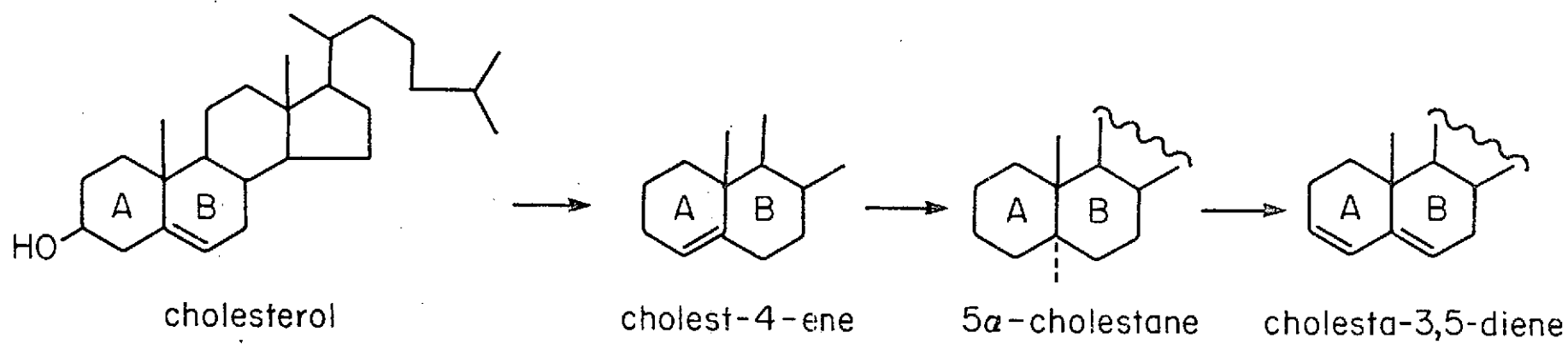
It is interesting to point out that about half of the original sterols survived heat treatment for 64 days (Table 4). There was some transformation of sterols into cycloaklanes, which is in accordance with the results of Rhead et al. (1971). GLC and MS study of the branched and cyclic hydrocarbons of the standard and heat-treated sediment revealed the presence of both steranes and sterenes of cholestane and probably of the ergostane and stigmastane series. In the standard sediment, both 5α - and 5β -cholestanes

were present, and 5α -predominating (3:1); a rather small quantity of 4- and 5-cholestenes was also detected. The existence of these cycloalkanes in the unheated sediment probably indicates preliminary microbiological degradation of the sedimented organic matter.

Heating the sediment at 150°C for two months increased the sterene content to a lesser extent than steranes. The dominance of 5α - over 5β -cholestane in the heated sediment is in accordance with the observation that catalytic reduction of cholesterol and related compounds yield the 5α - and 5β -isomers in proportions which depend on the temperature and the catalyst used (Adhikary and Harkness, 1969). A possible scheme for formation of sterenes and steranes of both α - and β -series proposed by Rhead et al. (1971). The following scheme describes the α series.

(Scheme 3)

It seems that thermal and reductive processes are of prime importance in formation of sterenes and steranes in the heat-treated sediment (Steel et al. 1972; Weiss 1969; Brooks and Smith 1969).



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FIGURE CAPTIONS

- Figure 1: (A) Total alcohol fraction of Tanner Basin sediment heated to 100°C, chromatographed as acetates on a 3% OV-101 column, programmed 100° to 300° at 4°/min.
- (B) Same sample after hydrogenation. Compounds labelled are: (a) unidentified peaks, (b) n-C₁₆OH, (c) Δ-n-C₁₈OH + dihydrophytol (d) n-C₁₈OH + phytol, (e) Δ-n-C₂₀OH, (f) n-C₂₀OH, (g) phthalate contaminant, (h) Δ-n-C₂₂OH, (i) n-C₂₂OH, (j) Δ-n-C₂₄OH, (k) n-C₂₄OH, (l) n-C₂₆OH, (m) 22-dehydrocholesterol, (n) cholesterol, (o) brassicasterol, (p) Δ⁷-ergosterol, (q) campesterol, (r) β-sitosterol

Table 1

Concentration (ppb) of C ₂₂ and C ₂₄ alcohols in heat-treated Tanner Basin sediment					
		65°	100°	150°	150° ±
	Standard	7 days	7 days	64 days	64 days
C ₂₂	350	350	620	n.d.*	180
C _{22:1}	250	450	1340	1820	1720
C ₂₄	240	500	440	n.d.*	200
C _{24:1}	190	970	2120	1850	1970
± freeze-dried prior to heating					
* n.d. = not determined					

Table 2. Concentration (ppb) and MS data for isoprenoid alcohols in Tanner sediment heat-treated for 30 days.

Compound	Temperature (°C)		Major m/e in MS (>100)
	100	150	
Phytol	374	142	111, 123, 278
Dihydrophytol	803	573	111, 125, 140, 183 196, 252, 280

Table 3. Relative abundance and molecular ions[±] in mass spectra of sterols extracted from Tanner Basin and Bandaras Bay sediment.

Assigned Structure	Abundance of sterols (%)		Molecular ion in MS [±]
	Tanner [*]	Bandaras ^{**}	
22-Dehydrocholesterol	18.7	tr.	366
Cholesterol	22.3	29.6	368
Brassicasterol	12.3	7.8	380
Δ ⁷ -Ergosterol	9.6	--	382
Campesterol	9.0	14.4	382
Stigmasterol	--	12.0	394
β-Sitosterol	28.1		396
		36.0 ⁺	
Triterpene	--		426

* Sample heated at 100°C for one week

** Sample heated at 150°C for one week

+ Inseparable mixture of β-sitosteryl acetate and a triterpene acetate, the latter appearing as a shoulder.

± Chromatographed as acetates. Molecular ion is actually M-60.

Table 4: Abundance (ppm) of cholesterol extracted from
Tanner Basin sediment during heating experiments[±]

Time (days)	Temperature (°C)		
	65	100	150
7	2.4	3.4	1.5
30	1.6	3.9	1.6
64	0.9	1.5	0.8

± = Cholesterol present initially in the sediment in the
concentration of 0.9 ppm

Tanner Sediment
heated 150°-8 days
Bound Fatty Acids

After hydrogenation

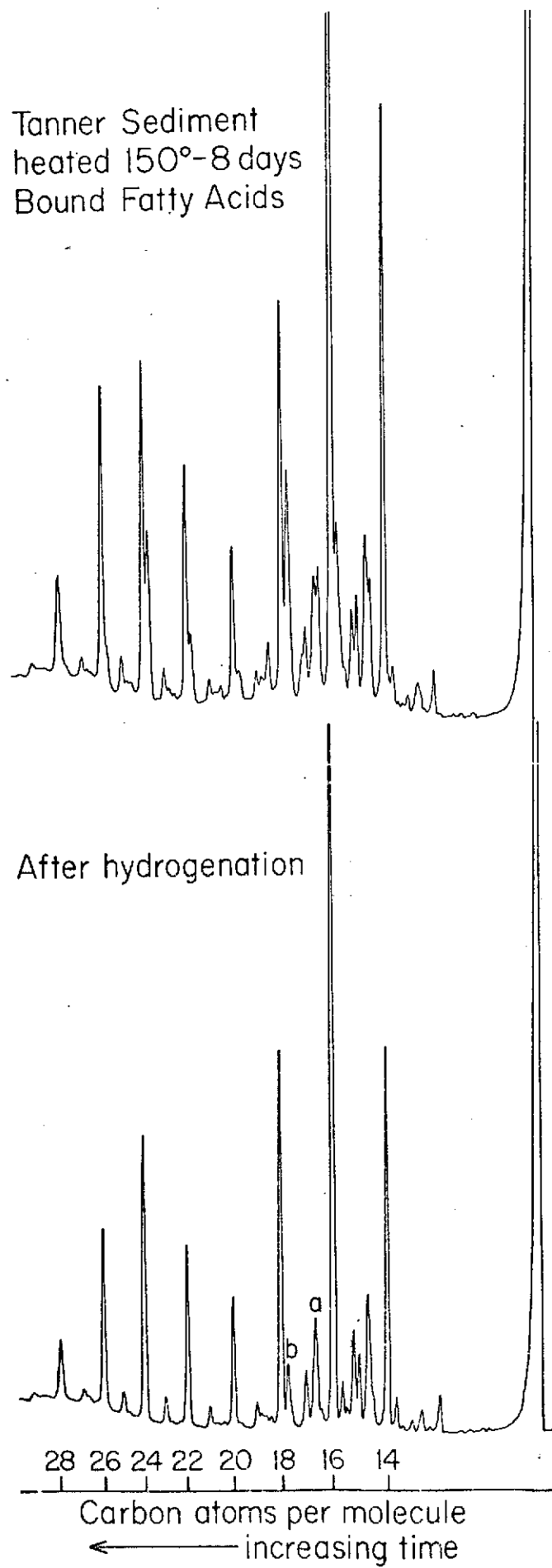


Fig. 1